Studies on Conformation, Structure, and Mechanical Properties of Copolypeptide Composed of γ–Benzyl-L-glutamate and ε–Carbobenzyloxy-L-lysine (Commemoration Issue Dedicated to Professor Keinosuke Kobayashi on the Occasion of His Retirement)

AUTHOR(S):

Hayashi, Toshio; Nakajima, Akio

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Studies on Conformation, Structure, and Mechanical Properties of Copolypeptide Composed of γ-Benzyl-L-glutamate and ε-Carbobenzyloxy-L-lysine

Toshio HAYASHI and Akio NAKAJIMA*

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Copolypeptides composed of γ-benzyl-L-glutamate (B) and ε-N-carbobenzyloxy-L-lysine (C) covering the whole range of copolymer composition were synthesized by the N-carboxyanhydride (NCA) method. From experimental results on the optical rotatory dispersion (ORD) regarding thermally induced coil-to-helix transition in dichloroacetic acid (DCA) -1,2-dichloroethane (DCE) system, it has been found that these copolypeptides can exist in the α-helix conformation in the same manner as homopolypeptides, PBLG and PCBL, in solution. The van't Hoff heat of transition, ΔH, showed a minimum against the initial monomer composition, suggesting the possibility of attribution to specific interactions between the side chain of one comonomer and that of the other comonomer in a two component copolymer. It is also pointed out that these copolymer molecules exist in the α-helix conformation in the solid state by infrared spectra measurements. The chain packing in unit cells for these copolypeptide films cast from solution has been investigated by wide angle X-ray diffraction patterns as a function of copolymer composition. The distance between adjacent chains was found to change linearly with copolymer composition. Thus, it may be concluded that cocrystallization of the two monomer units has occurred; *i.e.*, isomorphism is said to occur for the copolypeptides studied here over the complete composition range. The dynamic mechanical relaxation behavior of these copolypeptide films was also studied over the temperature range 0 to 200°C. From the relaxation behavior, it was considered that neighboring side-group interactions may be one of the important factors in determining side-group mobility, and this was seen to have a pronounced effect on the relaxation behavior of the copolypeptides.

INTRODUCTION

While rather extensive investigations have been conducted on the structure and the mechanical properties of homopolypeptide films or fibers,[1~16] very little information is available on the structure and the mechanical behaviors of copolypeptides. Hiltner *et al.*[17,18] have investigated the dynamic mechanical relaxation behaviors of copoly (γ-benzyl-L-glutamate/L-leucine) films cast from dioxane, as well as several homopolypeptides, and concluded that the neighboring side-group interactions might be an important factor in determining the side-group mobility.

In spite of many investigations on the dynamic mechanical properties in solid films of these polypeptides, comprehensive or systematic studies of relaxation or transition phenomena have not been performed in comparison with investigations on the superstructures obtained using X-ray and infrared techniques. Recently, Watanabe *et al.*[19] have

* 林　寿郎, 中島章夫 : Faculty of Engineering, Department of Polymer Chemistry, Kyoto University, Sakyo-ku, Kyoto, 606, Japan.

investigated the influence of casting conditions on the structure of PBLG, and showed that the effect of the casting solvent and temperature on the structure as well as mechanical properties were very notable.

In this paper, we are concerned with copolypeptides composed of γ-benzyl-L-glutamate(B) and ε-N-carbobenzyloxy-L-lysine(C). Three copolypeptide samples which have different comonomer ratios, as well as their related homopolymers, PBLG and PCBL, were synthesized by the N-carboxyanhydride (NCA) method, and the helix-to-coil transition behavior of these copolypeptides was investigated in solvents by measuring the optical rotatory dispersion (ORD). With these samples, in the next place, the molecular structure in solid state was analyzed as a function of copolymer composition by means of the infrared spectra (IR) and wide angle X-ray diffraction (WAXD) patterns. The dynamic mechanical relaxation behavior of these copolypeptide films as well as their related homopolymer films was also studied over the temperature range 0 to 200°C to make clear the effect of the neighboring side-group interactions on the copolypeptide properties.

EXPERIMENTAL

Materials

The monomers, N-carboxy-γ-benzyl-L-glutamate anhydride (BLG-NCA) and N-carboxy-ε-N-carbobenzyloxy-L-lysine anhydride (CBL-NCA) were prepared according to the method proposed by Blout and Karlson,[20] and purified by repeated recrystallizations from an ethyl acetate solution with the addition of petroleum ether. The BLG-NCA and CBL-NCA, in desired mole ratios, were dissolved in a 1 : 1 (v/v) mixture of dry dioxane and methylene dichloride. The total concentration of both anhydrides was kept at 2%. The polymerization was initiated with triethylamine (TEA) at an anhydride-to-initiator ratio ([M]/[I]) of 50. The polymerization was stopped at about 42 to 52 mol-% of conversion, monitored by the titration of the carbon dioxide evolved. All solvents used for synthesis and the initiator were purified more than three times by the usual methods described in the literature. The copolypeptides as well as homopolymers were precipitated in a large amount of cold methanol and dried under reduced pressure at 50°C. The composition of these copolypeptides was determined from elemental analysis and amino acid analysis. These results of all the copolymerizations are listed in Table I.

Measurements

The intrinsic viscosity, [η] (dl/g), of these samples was determined in dichloroacetic

Table I. Copolymerization of γ-Benzyl-L-glutamate (B) with ε-N-Carbobenzyloxy-L-lysine (C) by the NCA Method

Sample No.	Initial monomer ratio (G mol-%)	Polymer composition (G mol-%)	Conversion (%)	[η] (DCA, 25°C) (dl/g)
1	100	100	72	1.90
2	79	82	42	1.52
3	43	51	45	1.25
4	26	32	52	1.45
5	0	0	80	1.98

acid (DCA) at 25°C using Ubbelohde type capillary viscometers. Data are summarized in Table I.

ORD in a temperature range from 10 to 60°C was measured with a Yanagimoto OR-100 Type spectropolarimeter using a tungsten lamp as light source. The concentration of copolymer solution was 1.0 g/dl throughout these measurements. The solvent system used was a mixture of DCA-DCE.

IR spectra of solid films of the samples cast from chloroform solution were measured with a Perkin-Elmer Model 521 spectrophotometer in the region of 400–4000 cm^{-1}.

For the mechanical studies, films about 100 μ in thickness were cast onto plane glass plates from approximately 2% solutions in chloroform (CF) or benzene (Bz). Any residual solvent in the air-dried films was removed by treatment with ether and methanol. The films were then dried in vacuo for 3 days at room temperature prior to using.

WAXD profiles were obtained with a GE diffractometer, setting a flat surface of the film parallel to a reflecting surface with an automatic diffractometer.

The dynamic mechanical relaxation behavior was measured with a DDV-II Rheovibron at a frequency of 110 Hz and heating rate of 0.3°C per minute over the temperature range 0 to 200°C.

RESULTS AND DISCUSSION

Effect of Solvent Composition and Temperature on Molecular Conformations of Copoly (γ-benzyle-L-glutamate/ε-N-carbobenzyloxy-L-lysine).

The ORD of these copolypeptides was measured in DCA-DCE mixtures. The results are examined in terms of parameters appeared in the Moffitt equation:[21]

$$[\alpha]=\left(\frac{100}{M_0}\right)\left(\frac{n^2+2}{3}\right)\left[\frac{a_0\lambda_0^2}{\lambda^2-\lambda_0^2}+\frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2}\right] \tag{1}$$

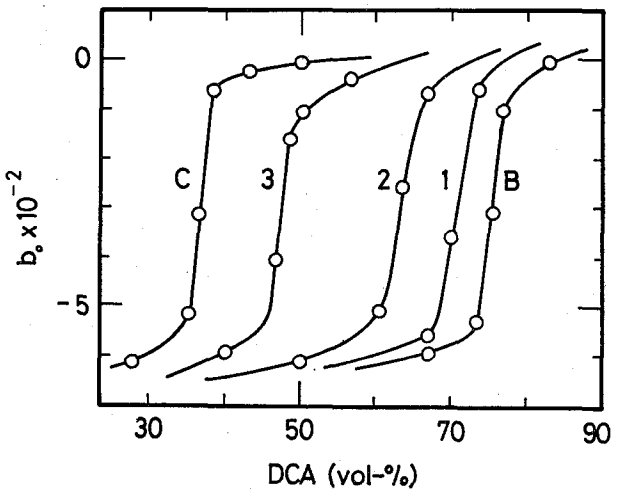

Fig. 1. Effect of solvent composition on coil-to-helix transition at 25°C in DCA–DCE mixtures; (B) PBLG-1, (1) BC-1 (82 mol-% of BLG), (2) BC-2 (51 mol-% of BLG), (3) BC-3 (32 mol-% of BLG), and (C) PCBL-5. The polymer concentration is 1.0 g/dl.

where a_0 is a constant which may very with the nature of the side chain of polypeptide and depends on the kind of solvent, whereas the parameter b_0 is a function of the helix content. Further, M_0 is the molecular weight per peptide residue, n the refractive index of the solvent and λ (nm) the wavelength of the light source. In our previous analysis of the dispersion data on PBLG and PCBL in DCA-DCE mixture solvents in which the helix conformation is stable, we obtained $b_0=-600$ for PBLG and -550 for PCBL with $\lambda_0=212$ nm.[22] Figure 1 illustrates the dependence of b_0 on the solvent composition as represented by DCA mol-%, showing that different amounts of DCA are required to destroy helix conformations at 25°C. The amount of DCA to initiate the helix-to-coil transition is lowest (about 37%) for PCBL and is highest (about 75%) for PBLG. The amounts of DCA required to destroy the helix conformation of BC-copolypeptides fall between that of PCBL and PBLG in proportion to their mole ratios of two comonomers. This means that the transition of the copolypeptide takes place not independently but cooperatively with respect to two comonomers.

Nextly, the conformational behavior of these copolypeptides resulting from change in temperature was investigated in DCA-DCE system. Since these homopolypeptides, PBLG and PCBL, are rather different in helix stability in solvent, as shown in Fig. 1, it was impossible to use the fixed composition of solvent mixture to compare the thermal transition curves of these copolypeptides. In order to keep the transition within an available temperature range, different solvent compositions were used. It was shown that the sharpness of transition was quite different from case to case, exactly similar behavior to our previous work for the same system (*i*.e., copoly(γ-benzyl-L-glutamate/ε-N-carbobenzyloxy-L-lysine)) composed of different molar ratio of two comonomers.[23] ΔH, the van't Hoff heat of transition, is determined experimentally from the slope of the b_0 versus temperature curve using the following equation:[24]

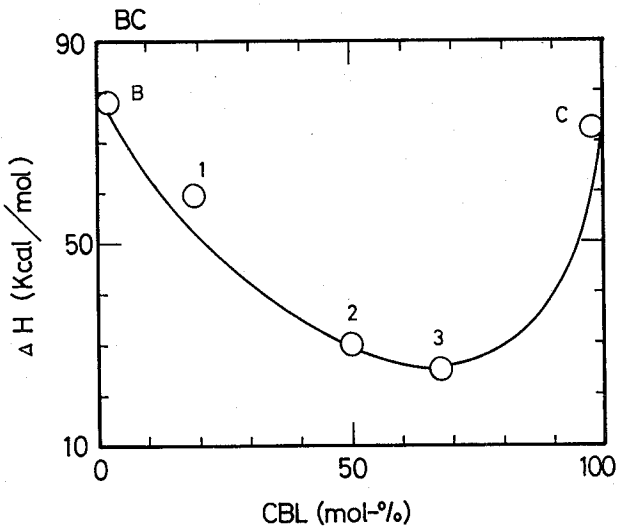

Fig. 2. van't Hoff heat of transition, ΔH, for thermal transition of BC-copolypeptides in DCA-DCE mixture as a function of mol-% of CBL component. The polymer concentration is 1.0 g/dl.

$$\Delta H = 4RT_t^2 \cdot \frac{1}{b_{0,h} - b_{0,c}} \cdot \left(\frac{db_0}{dT}\right) T_t \qquad (2)$$

The ΔH calculated for these copolypeptides as well as their related homopolypeptides are illustrated in Fig. 2 as a function of the CBL comonomer composition (mol-%). $b_{0,h}$ was assigned -630 from ORD measurements in DCE at 25°C,[22] while $b_{0,c}$ was assigned 0 from ones in DCA at 25°C,[22] both for PBLG.

It should be pointed out that the ΔH showed a pronounced minimum against the CBL comonomer composition, at around 70 mol-% of CBL. The similar behavior was also reported for copoly(γ-methyl-L-glutamate/ε-N-carbobenzyloxy-L-lysine)[25] and copoly-

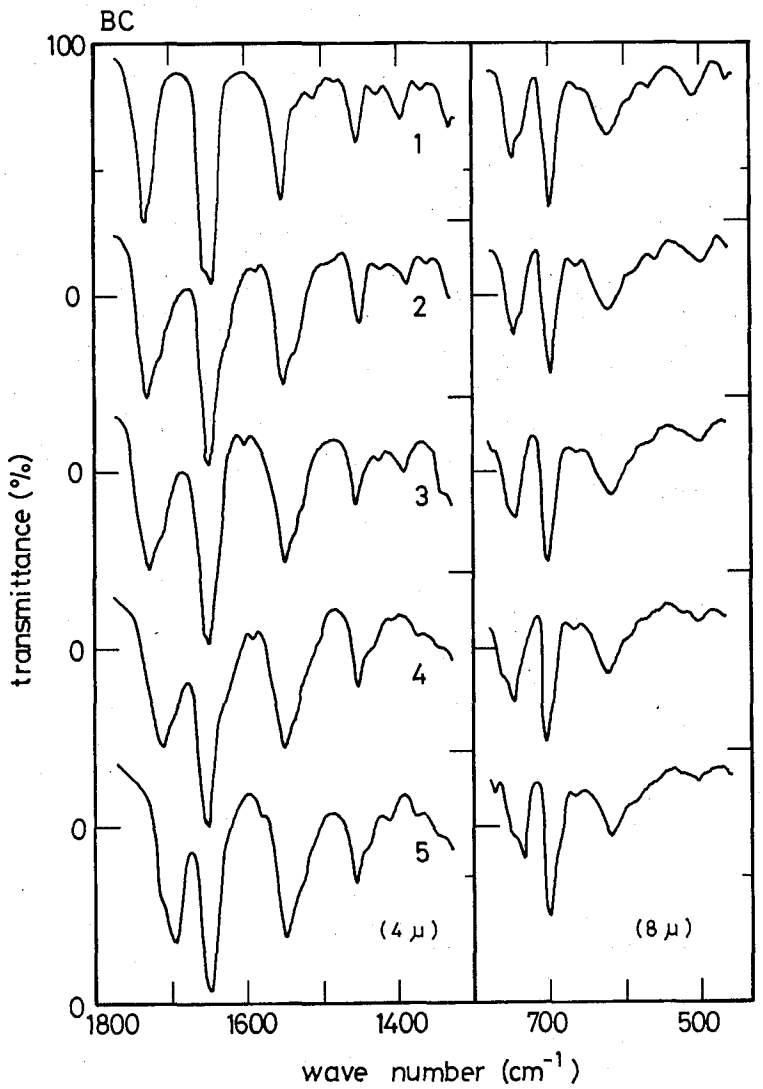

Fig. 3. Infrared spectra of unoriented solid films of BC-copolypeptides which were cast from chloroform solution at 25°C; (1) PBLG-1, (2) BC-1 (82 mol-% of BLG), (3) BC-2 (51 mol-% of BLG), (4) BC-3 (32 mol-% of BLG), and (5) PCBL-5.

(γ-benzyl-L-glutamate/γ-methyl-L-glutamate).[26] The presence of a minimum in these relationship may be attributed to specific interactions between the side chain of one comonomer and that of the other comonomer in a two component copolymer.

Chain Conformation of Copolypeptides in Solid State

IR spectra were measured with solid films of copolypeptides and their related homopolypeptides cast from chloroform solution. These spectra in the region of 1800–1300 and 800–500 cm^{-1} were shown in Fig. 3. The correlation of the amide I and II bands with chain conformation is now fairly well established,[27] and the amide I band, due to C=O stretching vibration, and amide II band, associated with C-N stretching and N-H deformation, of the α-helix conformation are expected to appear at 1650 and 1550 $cm,^{-1}$ respectively. Of the low-frequency modes, the amide V vibration has been most useful in structural investigations. It involves N-H out-of-plane bending and depends considerably on the backbone conformation. The α-helical and disordered forms may now be distinguished even for unoriented films, and the fraction of the α-helical form may be estimated in the presence of the disordered form. According to Miyazawa *et al.*[28] the amide V band of the α-helix appears at 610–620 cm^{-1} for PBLG and PCBL, while that of the disordered form at 650 $cm.^{-1}$ As is clear from Fig. 3, bands of amide I, II, and V for these copolypeptides appeared at 1650, 1550, and 615 $cm,^{-1}$ respectively, just at the same wavenumbers for both homopolypeptides, with almost the same order of peak intensity as those of PBLG and PCBL. Such a result means that these copolypeptides exist in helical conformation and, moreover, the helix content of these copolypeptides is nearly the same as that of homopolypeptides.

Wide Angle X-Ray Diffraction

The wide angle X-ray diffraction (WAXD) for the BC-copolymers, as well as the corresponding homopolymers, was investigated and the measured spacings are summarized in Table II. The pattern observed for homopolymer PBLG depends on the casting solvent and varies from the sharp, intence reflections of the chloroform cast film to the diffuse reflections produced by the benzene film. The effectiveness of the solvents in promoting crystallinity as determined from the WAXD patterns is in the order; chloroform>dioxane >benzene. The first main reflection corresponds to an intermolecular spacing of the α-helical chains, and has a spacing of 12.8 Å for the film cast from chloroform, and 13.2 and 13.3 Å for films from dioxane and benzene. Using the notation of McKinnon *et al.*,[29]

Table II. Wide Angle X-ray Diffraction Profiles of Copolymer Films Cast from Chloroform at 25°C

Sample No.	f_G	Equatorial Spacings (Å)				
1	1.00	12.8 *vs*	7.4 s	6.4 m	5.3 w	4.3 w
	1.00(DO)	13.2 *vs*	7.5 m	6.5 w	5.3 m	4.3 w
	1.00(Bz)	13.3 s	7.6 m	6.6 w	5.2 w	4.3 vw
2	0.82	13.2 *vs*	7.6 s	6.6 m	5.3 w	4.3 w
3	0.51	13.7 *vs*	8.0 s	6.8 m	5.2 w	4.3 vw
4	0.32	14.0 *vs*	8.1 s	7.1 m	5.3 w	4.3 vw
5	0.00	14.3 *vs*	8.3 s	7.2 m	5.3 w	4.3 vw

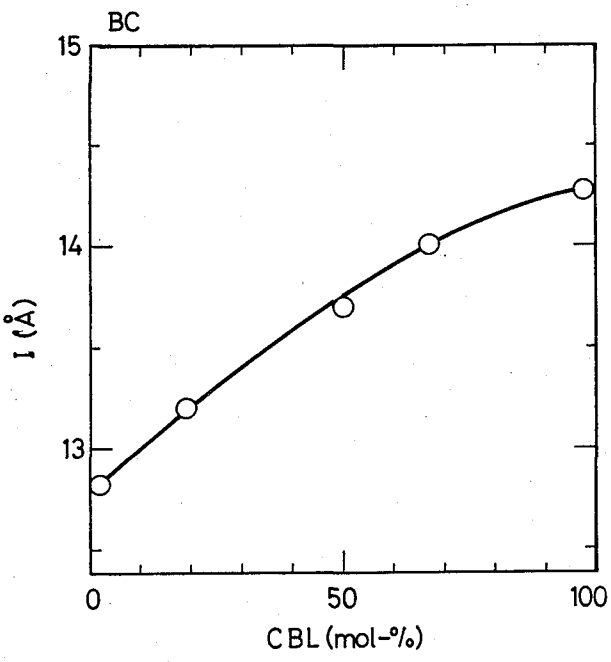

Fig. 4. First Equatorial Spacings for BC-copolypeptide films cast from chloroform solution as a function of mol-% of CBL component.

Table III. Distance between Adjacent Helices, a (Å), Calculated from X-ray Data Assuming the Hexagonal Unit Cell, and the Crystallographic Density

Sample No.	f_G	d_{obs}(Å)	a_{calc}(Å)	ρ
1	1.00	12.8	14.8	1.29
2	0.82	13.2	15.2	1.26
3	0.51	13.7	15.8	1.24
4	0.32	14.0	16.2	1.23
5	0.00	14.3	16.5	1.25

the film cast from chloroform is assigned the Form C solid state modification which has a crystalline structure equivalent to the unit cell described by Bamford.[30] Films cast from dioxane and benzene have the Form A structure, which is poorly ordered array thought to include superhelical aggregates of PBLG molecules.[31] Figure 4 illustrates the first equatorial spacings for BC-copolymer films cast from chloroform solution as a function of mol-% of CBL component.

The actual interhelical spacing is assumed to be equal to the unit cell dimension in the a-axis, and determined from (110) spacing for hexagonal unit cell.[32] The observed spacing and the calculated distance between adjacent helices are summarized in Table III. In Table III, also, are included the crystallographic densities, ρ, calculated from the volume, V (in Å^3), of the unit cell and the number, n, of molecules of molecular weight M in the unit cell by using the equation; $\rho=1.65\ Mn/V$. The density values of copolymers are slightly lower than those of homopolymers though the difference are not great. This means that the degrees of packing for copolymers are slightly looser than those of homo-

polymers.

The identity period along the chain in the same for PBLG and PCBL. Further, both homopolymers do not significantly differ in their crystal systems. From the result given in Table III, it may be concluded that cocrystallization of the two monomer units has occurred. In other words, isomorphism is said to occur for the copolypeptides studied here over the complete composition range. Additional discussion on isomorphic replacement will be given nextly, from a stand point of the copolymer composition dependence on the dynamic mechanical property.

Dynamic Mechanical Spectroscopy

Solvent-dependent structure modifications of PBLG previously characterized by the WAXD patterns also produce significant differences in the dynamic mechanical behavior. The loss spectrum, tanδ, of PBLG cast from chloroform solution shows a large β-peak at 42°C (110 cps) with a much weaker α-peak at 120°C (Fig. 5, B-1). As the apparent crystallinity is decreased by changing casting solvent in the order chloroform, dioxane, and benzene, the intensity of the β-peak decreases and that of the α-peak increased for the

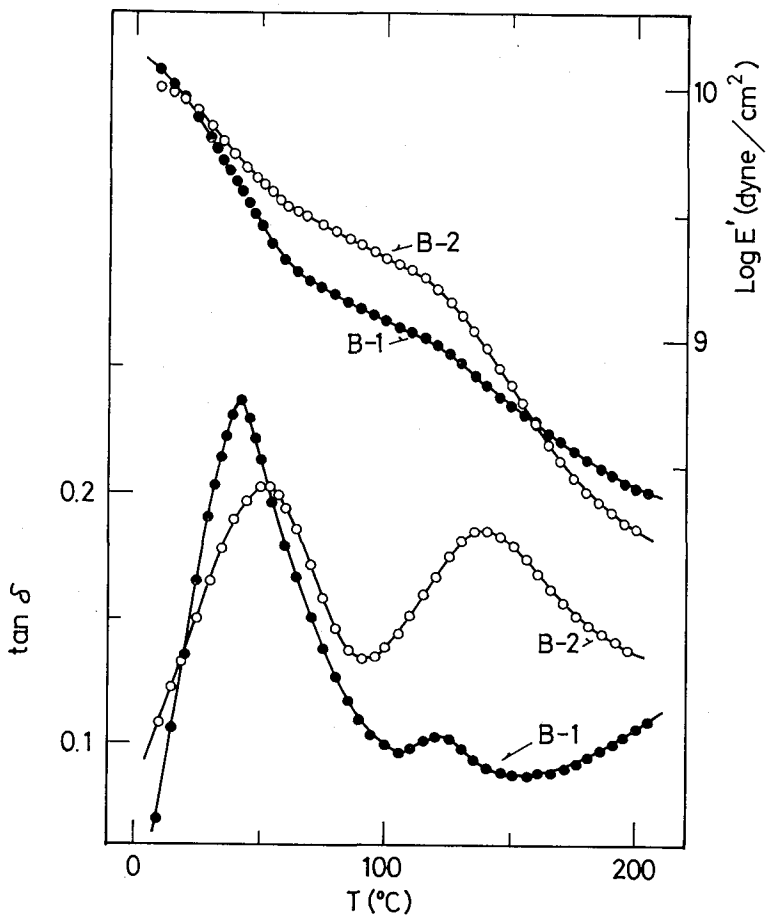

Fig. 5. Temperature dependence of tanδ and E′ for PBLG-1 films cast from; (B-1) chloroform and (B-2)benzene at 25°C.

film cast from benzene (Fig. 5, B-2). Similar results have been observed by Fukuzawa *et al.*[31] The change in the α-peak is considerably more pronounced, and for the benzene cast film the intensities of the β and α peaks are almost comparable. Small temperature shifts are also noted; the β is observed at 42° and 50°C, and the α at 120° and 140°C, respectively.

The storage modulus curves, E′, show a similar solvent dependency. A fairly large modulus drop occurs at the β process for the chloroform cast film followed by a smaller drop at the α temperature. The magnitude of the former decreases and the latter increases as the crystallinity decreases, and for the benzene cast film the largest drop is accompanied with the α process. The α process is generally attributed to the onset of motion of chain segments which are in a disordered conformation.[33] The increase in α intensity with decreasing crystallinity is consistent with this interpretation. The β process, which is associated with motion of the side chains, appears to include a strong crystalline contribution since the intensity decreases significantly in the less crystalline specimens. The

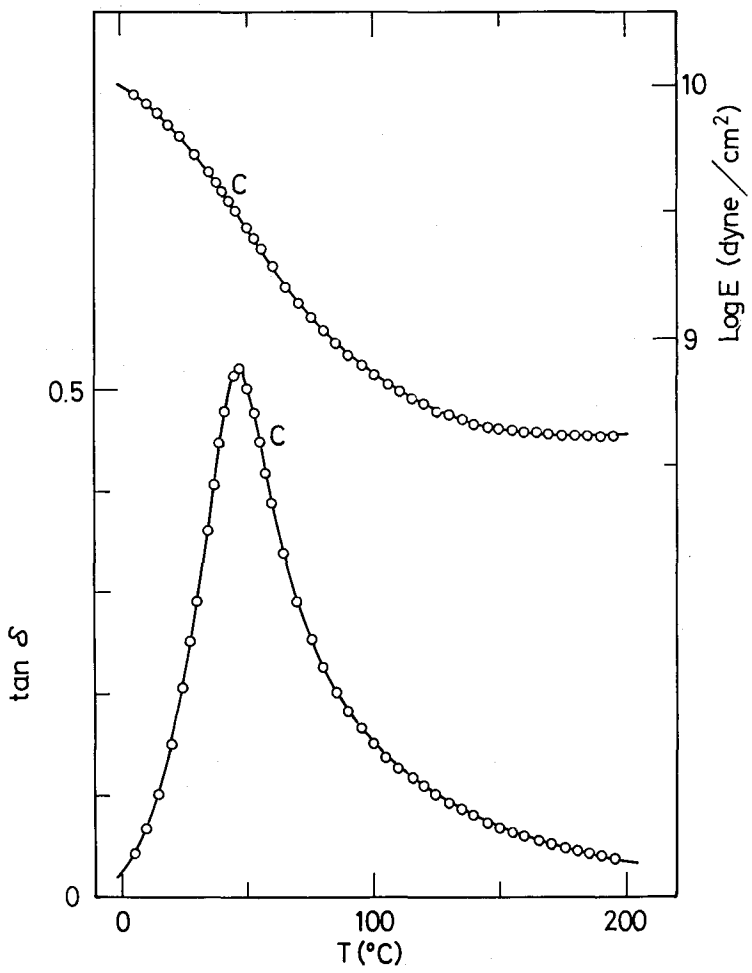

Fig. 6. Temperature dependence of tanδ and E′ for PCBL-5 film cast from chloroform at 25°C.

effect of crystallinity is particularly apparent in the modulus drop which is accompanied with the β process where comparison of B-1 and B-2 in Fig. 5 shows that in the plateau region between the β and α relaxations the modulus of the chloroform cast film lies well below that of the benzene cast film. If the side chains is assumed to be in ordered positions in the crystalline lattice as suggested by Fukuzawa *et al.*[31] the β process should be interpreted as a side chain melting. However, it is evident from DSC[34] and dielectric relaxation studies[2] that the β process more closely resembles a glass-rubber transition. A consistent hypothesis is that the side chains do not crystallize in the solid state, but constitute a relatively disordered material filling the lattice space between ordered, helical backbones. However, it is not clear why the β process should have a larger effect on the crystalline properties. Possibly, intermolecular effects are important, in which case the extended

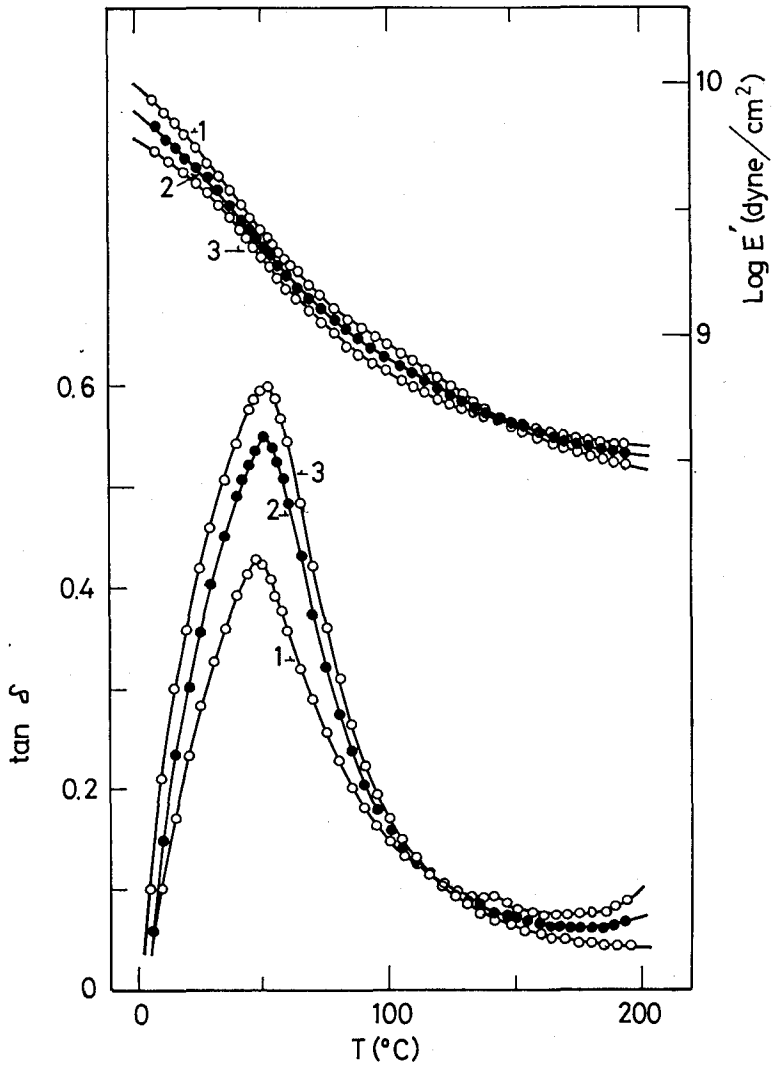

Fig. 7. Temperature dependence of tanδ and E′ for BC-copolypeptide films cast from chloroform at 25°C for; (1) BC-1 (82 mol-% of BLG), (2) BC-2 (51 mol-% of BLG), and (3) BC-3 (32 mol-% of BLG).

and ordered arrangement of the rigid backbones in the crystal could facilitate cooperative motion of the side chains and provide less resistance to intermolecular slip.

Figure 6 illustrates the temperature dependence of tanδ and E′ for PCBL homopolymer film cast from chloroform. This polymer shows a very strong β-relaxation peak at 47°C which is accompanied by a very sharp drop in modulus around the same temperature region. Hiltner *et al.*[17] reported the similar relaxation behavior for PCBL homopolymer film cast from dioxane, though the temperature of β-peak was slightly different from us, and β-peak at 50°C. The intensity of the β peak for PCBL film (Fig. 6) is stronger than that for PBLG film (Fig. 5, B-1); that is, $\tan\delta_{Max}=0.24$ and 0.52 for PBLG and PCBL, respectively.

The relaxation behavior of the BC-copolymer films is shown in Fig. 7. The most striking feature is the manner in which the magnitude and the temperature of the β process are influenced by the copolymer composition. A very weak α peak was observed in only one copolymer, a copolymer of highest BLG content. The intensity of the β peak increases as the content of CBL component increases, while that of the modulus decreases, with increasing the content of CBL component in around the same temperature region.

Figure 8 illustrates the behavior of the dynamic mechanical relaxation parameters of

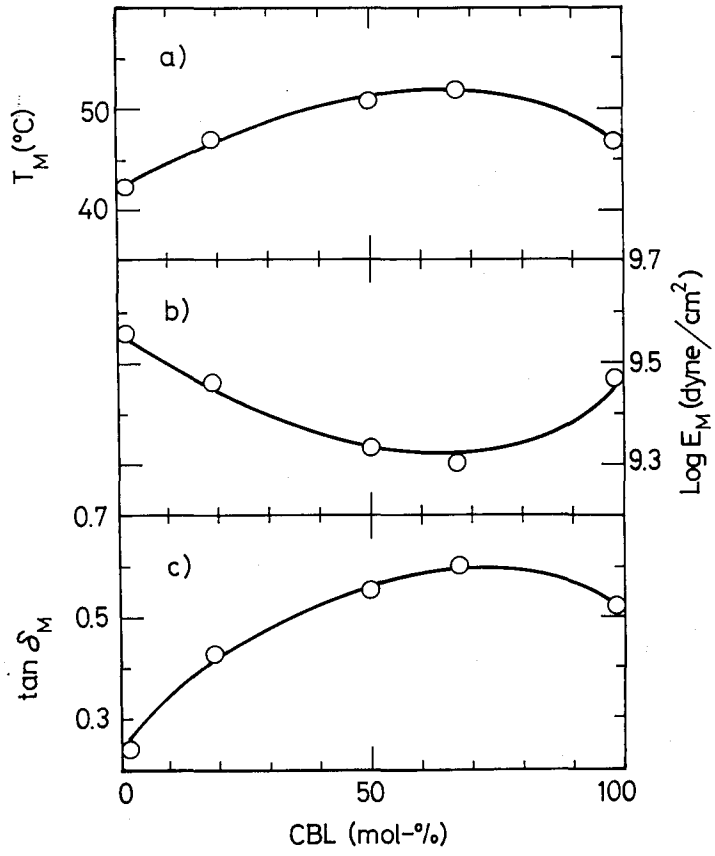

Fig. 8. The relaxation behavior of β-peak as a function of mol-% of CBL component in BC-copolypeptide films; (a) the temperature of β-peak, (b) the storage modulus, E′ (dyne/cm²) at T_M, and (c) the loss tangent, tan δ_M, at T_M.

β-peak as a function of mol-% of CBL component in BC-copolymer films; *i.e.,* the temperature of β-peak (T_M) (Fig. 8-a), the storage modulus, E′ (dyne/cm^2), at T_M (Fig. 8-b), and the loss tangent, tan δ_M, at T_M (Fig. 8-c). It is pointed out that these relaxation parameters are not exactly proportional to the CBL residue composition, but T_M and tan δ_M showed a maximum, while E′ a minimum against the CBL residue composition at around 70 mol-% of CBL. Further, it is notable that the value of this point is coincide not only with the minimum value of ΔH for the thermally induced coil-to-helix transition of BC-copolymers in DCA-DCE mixture (Fig. 2) but also the minimum value of ρ, the crystallographic density calculated from the X-ray parameters.

The reason for the non-linearity of the relaxation between these relaxation parameters and the mole fraction of comonomer residues should be attributed to the influence of the unique effect of the side chain-side chain interaction. If we consider the side chain-side chain interactions in α-helix conformation of polypeptides, there is strong packing of the side chain in homopolypeptides. While, in BC-copolymers, the condition of the packing of the side chains is highly modified by the unique side chain-side chain interactions. In this case, as the side chain groups contain the amide group, a unique interaction between the carboxyl group of BLG side chain and the amide group of CBL side chain seems to play an important role in the side chain-side chain interaction. Such neighboring side–group interactions may be also an important factor in determining the side-group mobility, and this seems to have a pronounced effect on the relaxation behavior of the copolypeptides in solid films.

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